COFRDA REPORT 3305

# Physiological and Biochemical Responses of Plants to Glyphosate: a Literature Review

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# Physiological and Biochemical Responses of Plants to Glyphosate: a Literature Review

**J.J. Zwiazek and T.J. Blake**
Faculty of Forestry
University of Toronto

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## ABSTRACT

Current literature on the broad-spectrum herbicide glyphosate [N-(phosphonomethyl)-glycine] is reviewed. The focus is on the uptake, metabolism and physiological effects of glyphosate, and on environmental influences on the herbicide's efficacy and mode of action, particularly as they relate to coniferous tolerance to the herbicide. It was found that glyphosate tolerance or susceptibility is a function mainly of uptake, translocation and protoplasmic tolerance, but varies widely among different species. This variation cannot be attributed to a single physiological or environmental factor. This points to the need for research on each species of interest to determine the factors most important for the response of that species to glyphosate.

## RÉSUMÉ

Les auteurs passent en revue la documentation actuelle sur le glyphosate [N-(phosphonomethyl)-glycine], un herbicide non sélectif. Ils s'attardent à l'absorption, au métabolisme et aux effets physiologiques du glyphosate et aux facteurs environnementaux influençant l'efficacité et le mode d'action de cet herbicide, notamment en ce qui concerne la tolérance des conifères à cet herbicide. Il a été découvert que la tolérance ou la sensibilité au glyphosate est principalement fonction de l'absorption, de la translocation et de la tolérance protoplasmique, mais varie énormément d'une essence à l'autre. Cette variation ne peut être attribuable à un seul facteur physiologique ou environnemental, laissant voir la nécessité d'effectuer d'autres recherches sur chaque essence en cause afin de déterminer les principaux facteurs intervenant dans les réactions de cette essence au glyphosate.

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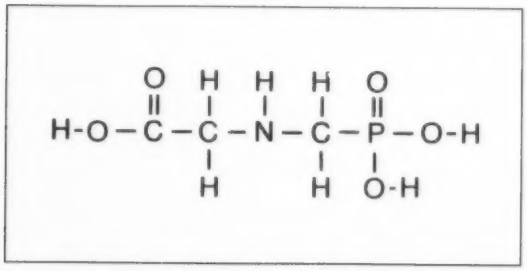
# PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF PLANTS TO GLYPHOSATE: A LITERATURE REVIEW

## INTRODUCTION

Glyphosate [N-(phosphonomethyl)-glycine] has been widely used as a herbicide since 1971, both in agriculture and in forestry. It has a relatively simple structure and easily forms water-soluble salts and derivatives, including isopropylamine salt, used in commercial preparations.

This literature review summarizes current knowledge concerning the effects of glyphosate on plants, based on a computer search using the BIOSIS reference source. The computer search yielded 25 titles for conifers and 163 titles for all plants. Approximately 40 of the most relevant papers are cited in this review. Older papers were obtained from reference lists found in newer research and review articles, for a total of 95 papers cited.



*Glyphosate [N-(phosphonomethyl)-glycine]*

Glyphosate appears to be environmentally safe because of its speed and pattern of degradation (Tate and Alexander 1974, Sprankle et al. 1975a) as well as its low toxicity to animals and people (Atkinson 1985). Glyphosate is used as a broad-spectrum, postemergence herbicide to control vegetation before and at the time of planting. It is particularly useful for control of perennial weeds because it is readily translocated to the roots, where it accumulates (Sandberg et al. 1980, Schultz and Burnside 1980). This prevents regrowth of shrubs and other perennial plants and results in their subsequent destruction.

The level of shrub control significantly affects establishment and growth of planted conifers. However, herbicide tolerance varies widely among coniferous species and individual trees of the same species (King and Radosevich 1985), and glyphosate can have negative effects on the growth of conifers. In the present report we attempt to summarize current knowledge about the mechanisms of the action of glyphosate on plants and the ways in which this herbicide can adversely affect growth of coniferous trees.

## ABSORPTION OF GLYPHOSATE BY PLANTS

Absorption and translocation of glyphosate in plants are the key factors determining the response of plants to this herbicide. The most economical, and therefore most frequently used, technique of glyphosate application is foliar spraying. The effect of foliar application depends on many factors, including the rate of glyphosate application, droplet size (Ambach and Ashford 1982, Buhler and Burnside 1987), concentration (Boerboom and Wyse 1988), the presence of adjuvants (Richard and Slife 1979, Sherrick et al. 1986, Bovey and Meyer 1987), soil moisture (Moosavi-Nia and Dore 1979a), rainfall (Bryson 1987, 1988), humidity (Wills 1978, McWhorter et al. 1980), light intensity (Moosavi-Nia and Dore 1979b, Schultz and Burnside 1980), growth stage (Neal et al. 1985), cuticular permeability (Wyrill and Burnside 1976), and plasmalemma permeability (Gottrup et al. 1976, Brecke and Duke 1980).

There is no agreement about the effect of temperature on glyphosate activity in plants. McWhorter et al. (1980) found that increased

temperatures resulted in increased absorption of glyphosate by johnsongrass (*Sorghum halepense*) and decreased absorption by soybean (*Glycine max*). On the other hand, Devine et al. (1983) showed that increasing the temperature had no effect on glyphosate absorption by quackgrass (*Agropyron repens*). Lowering the temperature also produces variable effects. Exposure of quackgrass to a temperature of -4°C had no effect on glyphosate toxicity, but when potato (*Solanum tuberosum*) plants were exposed to a low-temperature regime (13/4°C, day/night), glyphosate phytotoxicity was lower than in plants grown at higher temperatures (24/13°C) (Masiunas and Weller 1988).

## Season of Application

The season of application affects the susceptibility of plants to glyphosate in different ways. Glyphosate appears to have the least effect on conifers when sprayed in the fall (Ahrens 1974, Bing 1974) and dormant conifers can tolerate high doses of this herbicide (Lund-Hoie 1975, Ahrens 1981). On the other hand, deciduous trees are more susceptible to glyphosate applied in the fall than in the spring (Putnam 1976, Weller and Skroch 1983). Neal et al. (1985) examined in detail the effects of growth stage on glyphosate absorption and transport in ligustrum (*Ligustrum japonicum*) and blue Pacific juniper (*Juniperus conferta*). The authors showed that differences among species in overall tolerance, as well as in seasonal tolerance, of glyphosate were a result of differential absorption rather than of transport of the herbicide into the plant. Juniper plants absorbed significant amounts of $^{14}$C-glyphosate only when the herbicide was applied at the time of shoot elongation. Ligustrum absorbed relatively more $^{14}$C-glyphosate, mostly during bud-break and shoot termination.

Seasonal variation in glyphosate tolerance of ponderosa pine (*Pinus ponderosa*), Jeffrey pine (*Pinus jeffreyi*), sugar pine (*Pinus lambertiana*), Douglas-fir (*Pseudotsuga menziesii*), white fir (*Abies concolor*) and red fir (*Abies magnifica*) was described by Radosevich et al. (1980). These conifers were found to be more tolerant of herbicide applications after fall dormancy, with substantial injury and mortality occurring in response to spring and summer treatments. Severe injury caused by herbicide always occurred when photosynthetic rates were high, xylem water potentials were low and shoots were actively growing. The authors explained the seasonal nature of herbicide activity in conifers as being the result of differential absorption through leaves and restricted distribution after absorption.

## Water Stress

The effects of growth rate and physiological status on injury attributable to herbicide application were studied in ponderosa pine and an associated shrub, greenleaf manzanita (*Arctostaphylos patula*), by Paley and Radosevich (1984). As in previous studies, the maximum damage to both species occurred after spring and summer applications of glyphosate. Damage caused by glyphosate was well correlated with growth rate and xylem potential in ponderosa pine, but only with xylem potential in greenleaf manzanita. The authors concluded that herbicide selectivity is greatest when pine has ceased growing, at a time when xylem water potential is low in pine and high in manzanita.

The relative tolerance of some water-stressed plants to glyphosate can probably be attributed to reduced uptake as a result of stress-induced stomatal closure. DeGennaro and Weller (1984) attempted to correlate the differential susceptibility of field bindweed (*Convolvulus arvensis*) biotypes to glyphosate with leaf-surface characteristics. The authors found no correlation between the differential response and the numbers of stomata and epidermal cells. However, they did not measure stomatal openings, and the gas-exchange rates may not have been the same in the different biotypes.

## Differences Among Species

Differences in the rates of glyphosate absorption by plants may explain differences in their

tolerance of this herbicide. Monocots are known to absorb glyphosate rapidly (Sprankle et al. 1975b) and they are generally very susceptible to glyphosate treatments. On the other hand, absorption by woody plants, including conifers, is generally slow (Lund-Hoie 1979). It is interesting that the amount of glyphosate absorbed by woody plants appears to be independent of the thickness of the leaf cuticle (Lund-Hoie 1976, 1979). Cuticle thickness and composition are known to be the major factors controlling the absorption of other polar herbicides (Kirkwood 1978). Lund-Hoie (1980) explained high glyphosate tolerance of conifers as resulting exclusively from restricted needle penetration, and many studies appear to support this hypothesis. Restricted needle penetration can explain why actively growing shoots of conifers are susceptible to glyphosate and why those factors that promote stomatal closure reduce the toxic effects of glyphosate. This hypothesis, however, does not explain the different pattern of absorption exhibited by other woody plants, as illustrated by the ligustrum example in the study by Neal et al. (1985). Clearly, more studies are required to explain the varying susceptibility of different types of woody plants to glyphosate.

## TRANSLOCATION OF GLYPHOSATE IN PLANTS

Glyphosate uptake by plants appears to be by passive diffusion and is dependent on concentration (Gougler and Geiger 1981). Uptake can be influenced by the chelating effects of certain mineral ions (Sandberg et al. 1980), but Burton and Balke (1988) found that glyphosate uptake by suspension-cultured potato cells was either unaffected or was induced by the presence of $CaSO_4$, $MgSO_4$, $MnSO_4$, $ZnSO_4$ and $CaCl_2$.

The binding of glyphosate to cellular components, including the plasmalemma and cell walls, is very weak (Richard and Slife 1979), and contributes to apoplastic movement of glyphosate. Hence, the distribution of glyphosate in plants is partly determined by those factors that influence plant transpiration (Gottrup et al. 1976, Lund-Hoie 1983). However, glyphosate in plants must be transported through the symplast; if not, it cannot be exported from leaves. Rapid translocation of glyphosate to roots indicates that it is transported through the phloem. Indeed, glyphosate was found to follow closely the "source to sink" pattern, but some glyphosate was also found to move from the phloem to the xylem and into the apoplast along with the transpiration stream (Dewey and Appleby 1983, Jachetta et al. 1986).

The "source to sink" pattern of glyphosate translocation indicates that the most actively growing parts of plants should accumulate most of the herbicide and be the first to sustain injury. A direct relationship between sink activity and $^{14}C$-glyphosate translocation was documented by Lolas and Coble (1980). Prasad (1983) showed that most of the $^{14}C$-glyphosate applied to speckled alder (*Alnus rugosa*) and white birch (*Betula papyrifera*) was concentrated in growing roots, active buds, and young foliage. Pereira and Crabtree (1986) showed that roots, immature rhizomes, and tubers of yellow nutsedge (*Cyperus esculentus*) accumulated two to three times more glyphosate than comparable mature tissues. Keeley et al. (1985), also working with yellow nutsedge, found that glyphosate was more readily transported to young, 2-week-old tubers than to those 4 and 6 weeks old. Translocation of $^{14}C$-glyphosate was also found to be less in older (15 to 20 cm tall) barnyard grass (*Echinochloa cruss-galli*) than in younger (5 to 10 cm tall) plants (Ahmadi et al. 1980). DeGennaro and Weller (1984) reported similar findings for field bindweed.

Water stress can also affect translocation of glyphosate in plants, thereby alleviating its toxic effects. Ahmadi et al. (1980) investigated the effects of water stress on the absorption and translocation of glyphosate in barnyard grass and found that both processes were affected by low soil-moisture levels. Unfortunately, the authors did not measure actual levels of water stress in the plants. Other factors that influence the transport of assimilates in plants are also likely to influence the phytotoxicity of glyphosate.

However, when Masiunas and Weller (1988) investigated the effects of low temperature and light on glyphosate absorption and translocation in potato, they found that low temperature affects absorption rather than translocation and that light has no effect on the toxicity of glyphosate.

In woody plants, considerable amounts of glyphosate are transported in the transpiration stream to the places of most rapid transpiration, and from there glyphosate is retranslocated to the meristems of the youngest shoots (Lund-Hoie 1985). As a consequence of this distribution pattern, the first symptoms of injury caused by glyphosate in conifers appear in the top shoots and in those shoots that are actively growing. The author explained the seasonal activity in conifers as resulting from the differential absorption of glyphosate through leaves and restricted redistribution after absorption.

## METABOLISM OF GLYPHOSATE IN PLANTS

Few studies have been undertaken to investigate glyphosate metabolism in plants, although the subject has been thoroughly reviewed by Coupland (1985). Glyphosate is metabolized very slowly in higher plants and forms various metabolites or degradation products.

Rueppel et al. (1975a,b) showed that the main metabolic breakdown reaction in corn (*Zea mays*), soybean and wheat (*Triticum aestivum*) plants is the splitting of the carbon bonds through hydrolysis and decarboxylation. The authors proposed metabolic pathways of glyphosate in plants in which glyphosate is metabolized to glyoxylate and aminomethylphosphonic acid. Glyoxylate is further degraded in the glyoxylic and citric-acid cycles and is metabolized into natural plant products, whereas aminomethylphosphonic acid is degraded to formylphosphonic acid which, in turn, is converted to formaldehyde, the substrate for a number of natural plant products.

The degradation of glyphosate to aminomethylphosphonic acid is well established for soil microorganisms (Rueppel et al. 1977), but there is some controversy about the importance of this process in higher plants. Some authors have found aminomethylphosphonic acid in glyphosate-treated plants (Lund-Hoie 1976, Putnam 1976, Sprankle et al. 1978, Sandberg et al. 1980), whereas others could find no evidence of glyphosate degradation (Coupland and Caseley 1979, Marquis et al. 1979, Devine and Bandeen 1983). It is possible that not all higher plants are capable of degrading glyphosate; alternatively, the presence of aminomethylphosphonic acid in some plants could be explained by root exudation of glyphosate, its degradation by soil microorganisms to aminomethylphosphonic acid and subsequent absorption of aminomethylphosphonic acid by the roots.

Woody species appear to have a more complex glyphosate metabolism than the one described above. Lund-Hoie (1976, 1979) detected seven metabolites in Norway spruce (*Picea abies*), ash (*Fraxinus excelsior*) and birch (*Betula verrucosa*). The author suggested that glyphosate is conjugated with natural plant products and transported in this form, with the metabolite slowly degraded back to glyphosate.

## BIOCHEMICAL EFFECTS OF GLYPHOSATE

### Synthesis of Aromatic Amino Acids

Studies on the mode of glyphosate action in plants were pioneered by Jaworski (1972). The author treated duckweed (*Lemna gibba*) plants with glyphosate and various amino acids and noticed that growth inhibition caused by the herbicide can be alleviated by the addition of L-phenylalanine to the nutrient medium. Treatments with other amino acids did not produce significant reversals of growth inhibition. Glyphosate treatment also resulted in a reduction in the endogenous phenylalanine levels in the free amino acid pool in *Lemna* and in the increase in total free amino acids. On the basis of these findings, the author suggested that glyphosate promotes protein breakdown and

inhibits the biosynthesis of aromatic amino acids by repressing the activity of chorismate mutase and/or prephenate dehydratase.

Although Jaworski's (1972) findings were supported by the results of many later studies, his conclusions, as well as the model for glyphosate action, have not been widely accepted and the enzymes proposed by the author have proven to be insensitive to glyphosate (Roisch and Lingens 1974). The glyphosate-induced reduction in free-pool sizes of aromatic acids has been observed in a variety of plant species, including soybean (Hoagland et al. 1979), buckwheat (*Fagopyrum esculentum*) (Hollander and Amrhein 1980), bean (*Phaseolus vulgaris*) (Shaner and Lyon 1980), wheat (*Triticum* sp.) (Nilsson 1977), and cultured cells of *Cryptomeria* and *Perilla* (Ishikura et al. 1986). Haderlie et al. (1977) found that glyphosate had no effect on the aromatic-acid content of cultured carrot (*Daucus* sp.) cells, but this may be a result of differences in the growing conditions of cells in suspension culture.

The reduction in free aromatic amino acids appears to be a major result of glyphosate action and numerous studies have confirmed that supplementary feeding of aromatic amino acids to plants alleviates the deleterious effects of glyphosate (Shaner and Lyon 1980, Ishikura et al. 1986, Creswell et al. 1988). However, in other studies, the addition of aromatic amino acids failed to alleviate glyphosate-induced symptoms despite a decrease in endogenous levels of aromatic amino acids (Duke and Hoagland 1978, Cole et al. 1980, Lee 1980). Evidently, either the responses vary among plants or else not all the effects produced by glyphosate are caused by the depletion of aromatic amino acids.

## PAL Activity

An alternative theory that may explain the phytotoxicity of glyphosate was proposed by Duke and Hoagland (1978). The authors suggested that lower levels of aromatic amino acids are a result of the induction of phenylalanine ammonia lyase (PAL) activity by glyphosate. They observed that glyphosate treatments of dark-grown maize seedlings resulted in an increase in the activity of extractable PAL 24 to 48 hours before a reduction in growth occurred. Similar increases in the *in vivo* PAL activity were observed in cultured *Perilla* cells (Ishikura and Takeshima 1984) and in soybean seedlings (Duke et al. 1980, Hoagland et al. 1979), but the induction could not be achieved in the *in vitro* tests. However, Hollander and Amrhein (1980) found that glyphosate had no effect on the activity of PAL extracted from buckwheat. Studies using PAL inhibitors to reverse the toxic effects of glyphosate have not been very successful. Duke et al. (1980) found only marginal reversal of glyphosate-induced growth inhibition in soybean when plants were treated with a PAL inhibitor, amonoxy-*a*-phenylproprionic acid, and Cole et al. (1980) found that PAL inhibitors had no effect on the toxicity of glyphosate. Therefore, it appears that glyphosate indirectly interferes with PAL and that the induction of PAL activity is not a major cause of the toxicity of glyphosate.

## EPSP Synthase Activity

Steinrucken and Amrhein (1980) observed that glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase), and they hypothesized that this inhibition is responsible for many of the observed metabolic changes in plants. The target enzyme of glyphosate, EPSP synthase, is a critical enzyme in the biosynthesis of aromatic amino acids, since it catalyzes the addition of the enolpyruvyl moiety of phosphoenolpyruvate to shikimate-3-phosphate. Inhibition of EPSP synthase activity would therefore prevent the synthesis of chorismate-derived aromatic amino acids and secondary metabolites.

## Phenolic Acids

In addition to lowering the levels of aromatic amino acids, the inhibition of EPSP synthase activity results in elevated levels of shikimic acid

(Amrhein et al. 1980, Berlin and Witte 1981). The levels of shikimic acids are often higher than might be expected because lowering the levels of products of the shikimic acid pathway results in a deregulation of carbon flow into the shikimic acid pathway (Jensen 1985).

Levels of other phenolic acids were also found to increase in glyphosate-treated plant cells. Ishikura et al. (1986) found that, in addition to shikimic acid, quinic acid accumulated in cultured cells of *Cryptomeria* and *Perilla* as a result of glyphosate treatment. Lydon and Duke (1988) reported increases in shikimic, protocatechoic, gallic and 4-hydroxy-benzoic acid levels in plants of several species treated with glyphosate, whereas levels of vanillic and syringic acids remained unchanged. Canal et al. (1987a) observed an increase in benzoic acids (gentisic, hydroxybenzoic, salicylic and vanillic) and a decrease in cinnamic acids in yellow nutsedge. The authors also measured PAL activity in plants treated with 0.1, 0.01 and 0.0001 M glyphosate and observed a decrease in activity only after the 0.01-M treatment. This indicated only a marginal involvement of PAL in the action of glyphosate.

Glyphosate can also inhibit the synthesis of other phenolic compounds. Ishikura et al. (1983) and Teramoto and Ishikura (1985) found that glyphosate treatments of *Cryptomeria* and *Perilla* cell cultures resulted in a marked suppression in the formation of flavans and caffeic acid, even at glyphosate levels that were only slightly inhibitory to cell growth. Levels of soluble proteins in *Cryptomeria* were not affected by a short-term 2-mM glyphosate treatment.

## Genetic Engineering and Tolerance to Glyphosate

Genetic manipulations that have produced glyphosate-tolerant plants provide more evidence that the inhibition of EPSP synthase may explain glyphosate toxicity. Shah et al. (1986) cloned a gene for petunia (*Petunia* sp.) EPSP synthase and used it to engineer glyphosate tolerance by fusing a cDNA clone to the cauliflower mosaic virus (CaMv) 35-S prometer; this resulted in substantial enhancement of EPSP synthase activity as a result of overproduction of the enzyme. Klee et al. (1987) cloned an *Arabidopsis thaliana* gene that encodes EPSP synthase to obtain glyphosate-tolerant plants. The *Arabidopsis* gene is highly homologous to the petunia gene within the mature enzyme and contains seven introns that are at exactly the same positions as those in petunia, but which are smaller, so that the size of the gene is reduced. The authors fused the gene to the CaMv 35-S prometer, reintroduced the chimeric gene into *Arabidopsis* and obtained overproduction of EPSP synthase, which led to glyphosate tolerance in transformed callus and plants.

Tissue-culture techniques have also been used to select for glyphosate-tolerant lines. Nafziger et al. (1984), Steinrucken et al. (1986) and Creswell et al. (1988) selected glyphosate-tolerant cells grown in suspension culture and correlated glyphosate tolerance with the overproduction of EPSP synthase. However, more studies are needed to confirm the pattern of inheritance and the stability of these genetic changes in regenerated plants.

More recently, glyphosate has been shown to be involved with yet another shikimic acid-pathway enzyme. Pinto et al. (1988) studied the effects of four glyphosate concentrations (0.5, 1.0, 1.5 and 2.0 mM) on the activity of 3-deoxy-*d*-arabino-heptulosonate-7-phosphate synthase (DAHP synthase) in potato cells grown in solution culture, and showed that all four concentrations markedly increased the *in vivo* activity of the enzyme by increasing the amount of the enzyme. The authors used the same glyphosate concentrations to study the *in vitro* effects on DAHP synthase and did not observe any change in the enzyme's activity. Therefore, the induction of DAHP synthase activity in response to the herbicide probably occurred indirectly, by the induction of *de novo* protein synthesis.

## Plant Growth Regulators

The two major metabolic changes induced by glyphosate are disruption of protein synthesis and

formation of secondary compounds. However, glyphosate has many other effects of potential importance to plants. One of them is the effect on indoleacetic acid (IAA) content. Lee (1982) and Lee et al. (1983) found that IAA levels in tobacco callus culture decreased in response to glyphosate treatments. The authors concluded that this decrease was the result of accelerated oxidative degradation of IAA and the formation of conjugates. In other studies it was found that IAA levels increased in glyphosate-treated plants (Canal et al. 1987b, Rajesekaran et al. 1987). Canal et al. (1987b) quantified IAA in yellow nutsedge leaves and attempted to correlate an increase in IAA with a rise in gentisic acid levels. The authors hypothesized that elevated levels of phenolic compounds in glyphosate-treated plants help to protect IAA from oxidation by inhibiting IAA oxidase activity. Although the authors were not able to measure IAA oxidase activity in extracts from control and treated plants, they found elevated IAA and gentisic acid levels and demonstrated *in vitro* that gentisic acid inhibits IAA oxidase isolated from nutsedge leaves. However, changes in IAA content can also be caused by a direct inhibition of IAA transport by glyphosate (Baur 1979) or by the interference of glyphosate-induced ethylene accumulation (Cole et al. 1983) with IAA transport.

### Photosynthesis

Glyphosate is considered to be a non-photosynthetic herbicide. Sprankle et al. (1975b) found no initial effect of glyphosate on photosynthetic rates in *Agropyron repens* until 72 hours after exposure. The assimilation rate of soybean leaf cells was also found to be relatively unaffected by glyphosate (Tymonko and Foy 1978). Podesta et al. (1987) also failed to show changes in phosphoenolpyruvate carboxylase activity in glyphosate-treated maize (*Zea mays*) leaves. On the other hand, Cole et al. (1983) showed a marked inhibition (at low glyphosate concentrations) and induction (at high concentrations) of $CO_2$ uptake by detached flax (*Linum usitatissimum*) cotyledons. Ireland et al. (1986) studied the effect of glyphosate on the kinetics of induction of photosynthesis in wheat leaves and determined that neither the rate of carbon fixation nor the level of chlorophyll emission was directly affected by glyphosate treatment. The authors concluded that glyphosate affects photosynthetic induction kinetics by an indirect modification of carbon metabolism.

A marked inhibition of net carbon exchange has been reported for sugar beet (*Beta vulgaris*) by Geiger et al. (1986, 1987). Geiger et al. (1987) showed that photosynthetic inhibition in sugar beet was not caused by changes in stomatal conductance because internal $CO_2$ levels were not affected by a glyphosate-induced reduction in stomatal conductance. The authors constructed two hypotheses: that glyphosate may inhibit regeneration of biphosphate carboxylase or that glyphosate limits ATP or NADPH production. Unfortunately, they presented no evidence in favor of either mechanism.

## CONCLUSIONS

Glyphosate is a potent herbicide that probably has more than one site of action in plants. The tolerance of some plants to glyphosate may be explained by (i) restricted uptake and translocation or (ii) protoplasmic tolerance. Evidence for the latter mechanism was found in those cells that overproduce EPSP synthase. The response of plants to glyphosate appears to be controlled by many factors and varies among plant species. Therefore, the conclusions reached in studies conducted with one species may not be valid for other plants. Few studies have been conducted on conifers; hence, little is known about how glyphosate interferes with metabolism in coniferous trees.

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